CX3CL1–CX3CR1 interaction mediates macrophage-mesothelial cross talk and promotes peritoneal fibrosis

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Peritoneal dialysis (PD) is limited by chronic fibrotic remodeling of the peritoneal wall, a transforming growth factor-β (TGF-β)-mediated process. The fractalkine (CX3CL1) receptor CX3CR1 is expressed on macrophages and monocytes, where it is a marker of TGFβ expression. Detection of its ligand CX3CL1 on the peritoneal mesothelium led us to hypothesize a pathophysiologic role of CX3CL1-CX3CR1 interaction in peritoneal fibrosis. We found that CX3CL1 was expressed on peritoneal mesothelial cells from PD patients and in a murine PD model. CX3CR1, mostly expressed on macrophages in the peritoneal wall, promoted fibrosis induced by chronic dialysate exposure in the mouse model. Our data suggest a positive feedback loop whereby direct interaction with CX3CR1-expressing macrophages promotes mesothelial expression of CX3CL1 and TGFβ expression. In turn, TGFβ upregulates CX3CR1 in murine and human monocytic cells. Upstream, macrophage cytokines including interleukin-1β (IL-1β) promote mesothelial CX3CR1 and TGFβ expression, providing a starting point for CX3CL1-CX3CR1 interaction. IL-1β expression was enhanced by exposure to dialysate both *in vitro* and in the mouse models. Our data suggest that macrophage-mesothelial cell crosstalk through CX3CR1-CX3CL1 interaction enhances mesothelial TGFβ production, promoting peritoneal fibrosis in response to dialysate exposure. This interaction could be a novel therapeutic target in PD-associated chronic peritoneal fibrosis.

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Translational Statement

The interaction of the stalked chemokine CX3CL1 on peritoneal mesothelium with its receptor CX3CR1 on macrophages promoted chronic inflammation and fibrosis in a peritoneal dialysis mouse model. Both the receptor and its ligand were detected on human peritoneal cells and similarly regulated, consistent with parallel mechanisms. Now more extensive human studies for association with peritoneal function should be undertaken. Therapeutically, our mechanistic data propose that i.p. extracellular inhibition of receptor-ligand interaction suffices to prevent its profibrotic effect, a hypothesis that needs to be tested with an appropriate agent starting in an animal setting.

Preservation of peritoneal transport and ultrafiltration capacity is a main challenge for improvement of peritoneal dialysis (PD), which is currently used as renal replacement therapy by approximately 11% of patients worldwide.[1,2] Risk factors for deterioration of peritoneal function are time on dialysis, glucose concentration in dialysis solutions, and peritonitis episodes.[3–6] The peritoneal wall contains a dense network of capillaries required for solute exchange and is covered by a layer of mesothelial cells.[7,8] This architecture is damaged in peritoneal fibrosis, a chronic transforming growth factor (TGF)-β–mediated remodeling.[3–6] However, how mesothelial cell interaction with other peritoneal wall cells modulates fibrosis is incompletely understood.

Macrophages are among the most common leukocytes in the resting peritoneal cavity.[9,10] Their numbers further increase in peritoneal fibrosis in humans and mice, and the cells become activated.[11] An increase of profibrotic macrophage subtypes[12] was reported in human PD effluents[13] and a mouse model of PD.[14] Leukocytes numbers increase not only in the cavity but also within the peritoneal membrane after short-term stimulation.[15,16] Inflammatory cytokines such as interleukin (IL)-1β[16,17] contribute to macrophage accumulation and effector functions in acute peritoneal inflammation. In contrast, much less is known about myeloid cell interaction with the mesothelium during chronic exposure to dialysis solution as it occurs in PD.

Fractalkine receptor CX3CR1 is a leukocyte surface protein that is most abundant on monocytes and macrophages in various organs.[18,19] It is differentially expressed on monocyte subtypes and regulated during human macrophage differentiation. Beyond a role in monocyte adhesion and recruitment, CX3CR1 is a marker of monocytic cells producing the profibrotic mediator TGF-β.[20] However, its mechanistic role in fibrosis is controversial, with profibrotic functions[21–24] and antifibrotic functions[25,26] depending on the organ and type of injury. For example, after muscle injury, complete CX3CR1 deficiency protected from necrosis, but ablation of $CX3CR1^{HIGH}$–expressing macrophages aggravated the injury.[27] Conversely, an increase in proinflammatory macrophages was detected in the absence of CX3CR1 in the majority of[26] but not in all[25,28] reports. CX3CR1 is also expressed on other leukocytes. We recently found that TGF-β upregulates CX3CR1 in T cells.[29] Similar TGF-β effects have been described in glial differentiation[30,31] and in mesangial cells.[32] Regulation by TGF-β in monocyte-derived macrophages and a mechanistic role of CX3CR1 in peritoneal fibrosis have not been reported.

The CX3CR1 ligand, the chemokine CX3CL1 (fractalkine), mostly has been investigated in endothelium, epithelium, and fibroblasts, where it was regulated by proinflammatory and profibrotic cytokines.[33,34] It exists in 2 forms, a soluble factor and a mucin-stalked cell-surface protein.[19] CX3CL1 has been detected in human peritoneal cells[35,36] and in the peritoneal cavity during bacterial peritonitis in mice.[37] Mechanisms of CX3CL1 regulation in the mesothelium and its impact in peritoneal fibrosis are not currently known.

The detection of peritoneal mesothelial CX3CL1 after chronic exposure to dialysis solution *in vivo* prompted this investigation of the regulation and pathophysiologic relevance of CX3CL1-CX3CR1 interaction in peritoneal fibrosis.

RESULTS

Peritoneal dialysis and proinflammatory cytokines increase peritoneal mesothelial CX3CL1 expression

To investigate regulation and role of CX3CL1 and its receptor CX3CR1 in PD-induced chronic fibrosis, wild-type mice underwent a 6-week course of daily peritoneal instillation of sterile, pyrogen-free PD solution for human use. A subcutaneous access port was used to avoid repeat damage to the peritoneal wall.[11] Identically treated CX3CR1-deficient ($CX3CR1^{-/-}$) mice on the same C57BL/6J background[38] served as controls. Body weight, spleen weight, and full blood counts were very similar in the groups (Supplementary Table S1).

In PD-treated mice, CX3CL1 was found on the mesothelial lining of the murine peritoneal cavity that stained for mesothelial markers (Figure 1a). In the absence of its receptor,[39] soluble CX3CL1 in the lavage was above the detection limit and its concentration significantly increased with PD (Figure 1b). PD fluid did not directly affect CX3CL1 production by mesothelial cells (Supplementary Figure S1). Therefore, the roles of cytokines previously described to modulate CX3CL1 in other cell types, namely IL-1β, tumor necrosis factor (TNF)-α, interferon-γ, and TGF-β[20,23,33,34] were investigated in mesothelial cells. TGF-β, IL-1β, TNF-α, or interferon-γ alone had little effect on CX3CL1 mRNA expression in murine mesothelial cells or endothelium used as positive control (Figure 1c and d). However, IL-1β and TNF-α combined significantly enhanced CX3CL1 expression. Combined cytokine effects were further amplified by TGF-β. CX3CL1 regulation in mesothelial cells was more marked than in endothelium, a cell type that has been intensely studied for this chemokine. Effects on fibroblasts as another possible CX3CL1 source in the peritoneal wall were even more moderate (Figure 1e). CX3CL1 protein secretion paralleled the mRNA results with strongest effects of IL-1β and TNF-α together with TGF-β in mesothelial cells (Figure 1f and g). Confocal microscopy of control and cytokine-stimulated peritoneal mesothelial cells was consistent with a parallel increase of CX3CL1 surface expression (Figure 1h).

The data identify peritoneal mesothelium as a significant source of CX3CL1 with a strong upregulation in response to inflammatory cytokine stimulation.

CX3CR1 promotes chronic peritoneal fibrosis

To test for a function of CX3CR1 in dialysis-associated chronic fibrosis, peritoneal thickness was assessed in mice exposed to PD solution (Figure 2a). After therapy, the peritoneum in wild-type mice was significantly thicker than in identically treated $CX3CR1^{-/-}$ controls by Masson's trichrome stain with an intermediate phenotype in $CX3CR1^{+/-}$ mice (Figure 2b). By picrosirius red collagen stain, no increase was observed if mice were treated with sterile normal saline solution after catheter implantation. However, in mice treated with PD solution, thickness increased significantly more in wild-type than in $CX3CR1^{-/-}$ animals (Figure 2c).

We investigated underlying mechanisms. The vast majority of $CX3CR1^{+}$ cells in the peritoneal wall were of myeloid origin, namely macrophages (Supplementary Figure S2). CX3CR1 modulates leukocyte numbers and cell death in some pathophysiologic situations.[19] Macrophage apoptosis induced by serum starvation was unaffected by TGF-β and CX3CL1 (Supplementary Figure S3). This outcome was very similar in wild-type and $CX3CR1^{-/-}$ cells, in agreement with published data.[20] Monocytic cell generation was studied next. Bone marrow myeloid progenitor numbers were very similar in resting and PD solution–exposed mice irrespective of CX3CR1 (Supplementary Figure S4). Bone marrow–derived macrophage proliferation was investigated in the presence and absence of TGF-β and CX3CL1 (Supplementary Figure S5). Wild-type macrophages proliferated more. This difference was unaffected by the cytokines. Also *in vivo,* Ki67 cell proliferation marker was expressed at higher levels in wild-type than in $CX3CR1^{-/-}$ blood monocytes upon exposure to PD solution (Supplementary Figure S6). However, this outcome did not translate to enhanced myeloid cell Ki67 in the peritoneal wall (Supplementary Figure S6C and F), and

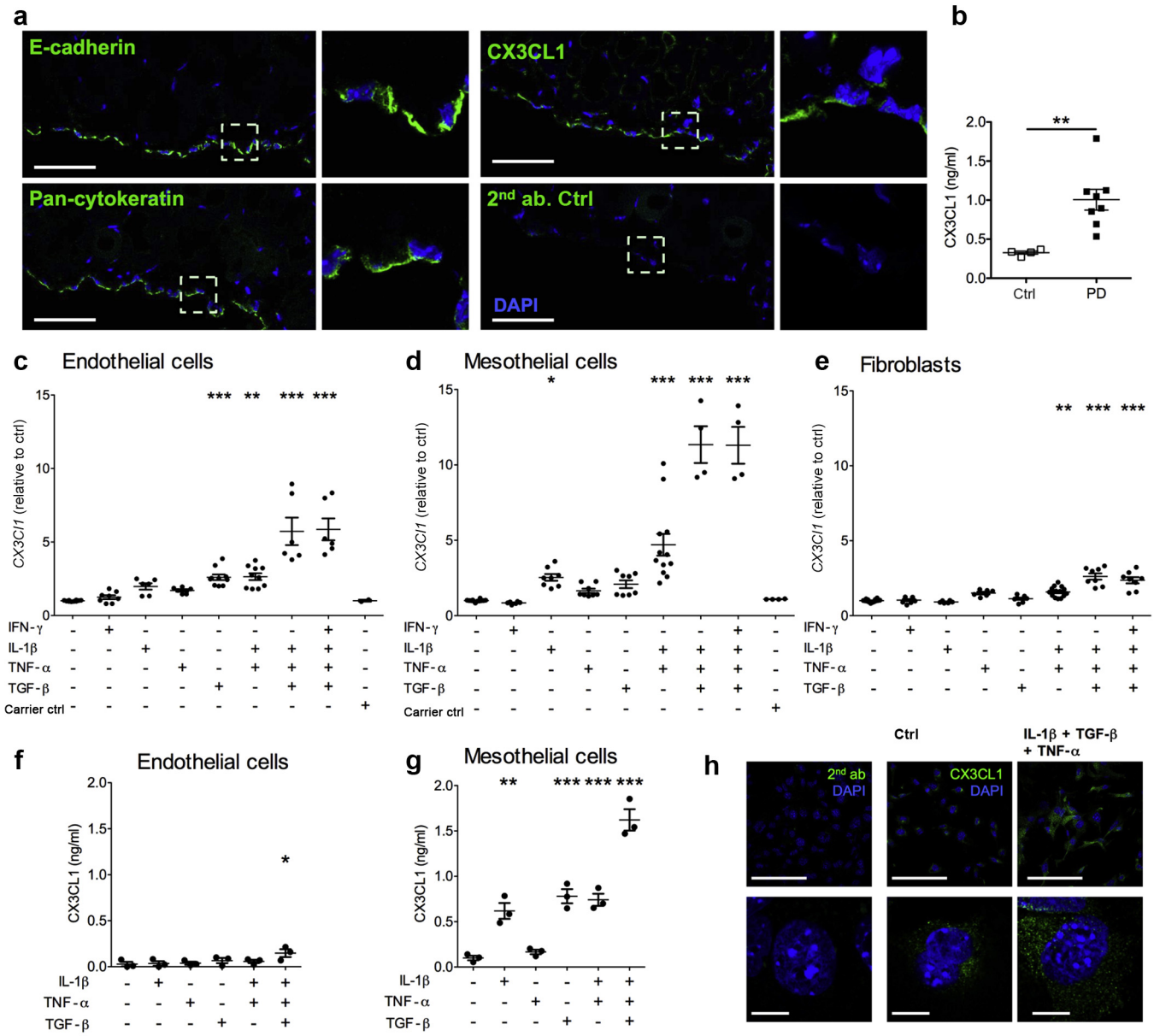

Figure 1 | Peritoneal mesothelial CX3CL1 is upregulated by peritoneal dialysis (PD) therapy and proinflammatory cytokines. (**a,b**) Wild-type and *CX3CR1*$^{-/-}$ mice were treated with peritoneal dialysis solution daily for 6 weeks. (**a**) Confocal microscopy of the peritoneal membrane after immunostaining with 4′,6-diamidino-2-phenylindole (DAPI) nuclear counterstain (blue, bar = 50 μm). CX3CL1 and mesothelial markers e-cadherin and pan-cytokeratin localized to the inner peritoneal layer of cells in PD-treated wild-type mice (green, typical examples, original magnification ×63). (**b**) CX3CL1 concentration was determined in the peritoneal lavage ($n = 4$ control [ctrl], $n = 8$ PD *CX3CR1*$^{-/-}$ mice). (**c–e**) Murine endothelial (**c**) and peritoneal mesothelial cells (**d**) and fibroblasts (**e**) were stimulated with interferon (IFN)-γ (10 ng/ml), interleukin (IL)-1β (10 ng/ml), tumor necrosis factor (TNF)–α (10 ng/ml), transforming growth factor (TGF)–β1 (10 ng/ml), combinations thereof, or carrier protein control. CX3CL1 mRNA expression was assessed after 4 hours (mouse cardiac endothelial cells, $n =$ 6–10 from 3–5 independent experiments; murine peritoneal mesothelial cells, $n = 4$–10 from 2–5 experiments; fibroblasts, $n = 8$ from 4 experiments; Dunn's tests after analysis of variance). (**f,g**) CX3CL1 concentrations in endothelial cell (**f**) and mesothelial cell (**g**) supernatants after 24 hours were determined by enzyme-linked immunosorbent assay after 4 hours of cytokine stimulation ($n = 3$ independent experiments). (**h**) Mesothelial cells with and without stimulation were stained for CX3CL1 (blue = DAPI nuclear stain, original magnification ×63, bars = 100 and 10 μm). ab, antibody. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

wall leukocyte numbers were independent of CX3CR1 after PD (Supplementary Figure S7) and also after initial exposure to PD solution (Supplementary Figure S8). Blood monocyte subpopulations differed between wild-type and *CX3CR*$^{-/-}$ mice. This phenotype was unaltered by PD (Supplementary Figure S9). Overall, these data suggest that other mechanisms than direct myeloid cell number changes are responsible for the increase in peritoneal fibrosis.

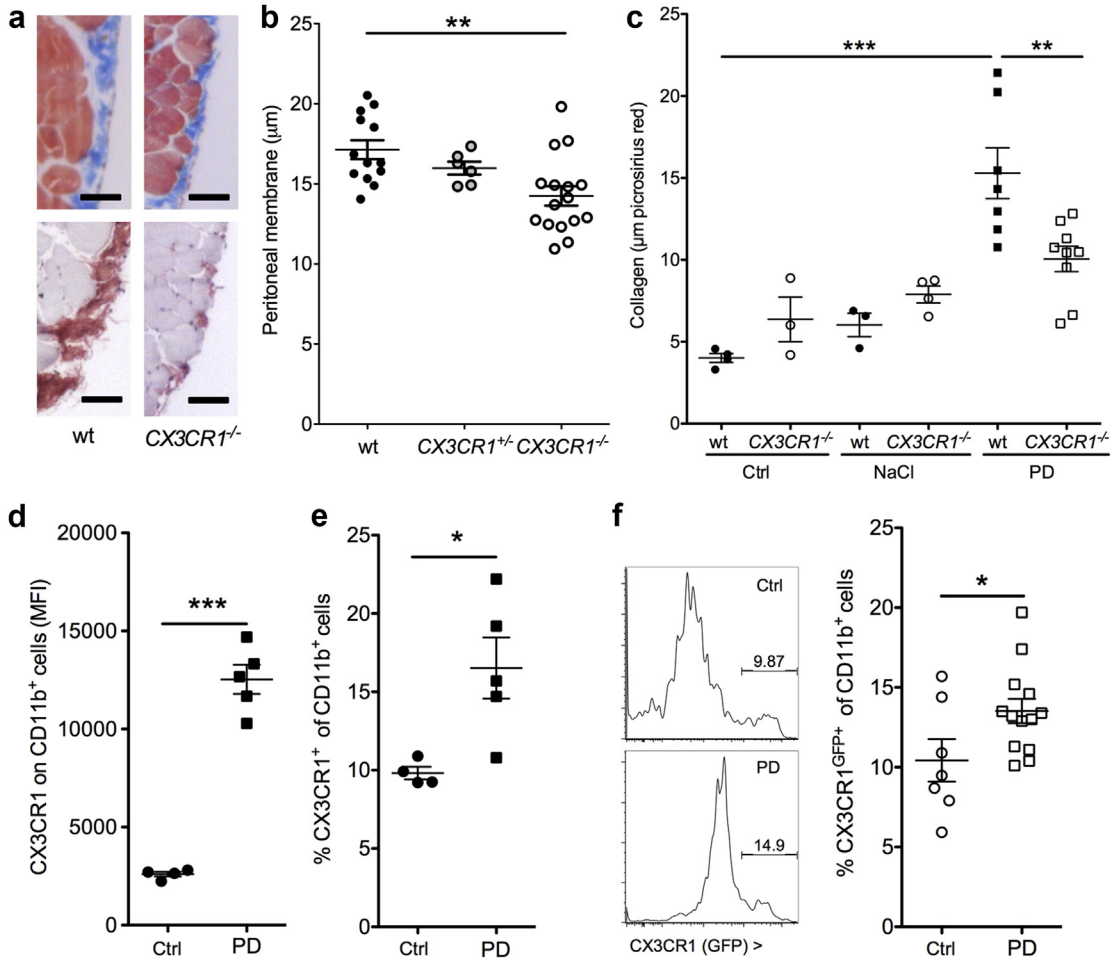

Figure 2 | The absence of CX3CR1 attenuates peritoneal dialysis (PD) fluid–induced chronic peritoneal fibrosis *in vivo*. (**a–f**) Wild-type (wt) *CX3CR1*$^{+/-}$ and *CX3CR1*$^{-/-}$ mice were treated with PD solution or control normal saline solution (NaCl) as indicated daily for 6 weeks. (**a–c**) Peritoneal membrane thickness was determined after Masson's trichrome and picrosirius red staining (**a**, examples, bars = 50 µm). (**b,c**) Statistical analysis of average per animal Masson's trichrome (**b**, Bonferroni after analysis of variance [ANOVA], $n = 6$, 13, 16 per genotype, 3–4 independent experiments) and of picrosirius red staining (**c**, $n = 3$–4 control [ctrl] and 7 and 9 PD animals, Bonferroni after ANOVA). (**d,e**) CX3CR1 surface expression on live inner peritoneal wall CD11b^{+} cells assessed by flow cytometry. Mean fluorescence intensity (MFI, **d**) and percentage of high-expressing cells (**e**) are shown for wt mice after PD and untreated ctrls ($n = 4$–5, *t*-tests). (**f**) The proportion of green fluorescent protein (GFP) reporter gene high-expressing cells was assessed in *CX3CR1*$^{GFP/GFP}$ reporter mice (*t*-tests, $n = 7$–12 from 3 independent experiments). To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

CX3CR1 expression level in individual cells may modulate their impact on disease. In animals treated with PD solution, CX3CR1 increased significantly in peritoneal wall macrophages (Figure 2d and e). A very similar result was obtained for CX3CR1 reporter gene expression (Figure 2f). This outcome occurred despite the fact that direct exposure to PD solution rather downregulated CX3CR1 expression in mouse bone marrow–derived and primary peritoneal macrophages *in vitro* (Supplementary Figure S10).

These data demonstrate a profibrotic function and upregulation of CX3CR1 in peritoneal fibrosis *in vivo*.

Fibroblasts and TGF-β upregulate CX3CR1 *in vitro*

Upregulation and the profibrotic role of fractalkine receptor CX3CR1 in the peritoneum *in vivo* prompted us to investigate possible mediators of CX3CR1 regulation in fibrosis. To test the role of fibroblasts as putative CX3CR1 upregulators, co-culture with macrophages was used. In direct and transwell co-culture, fibroblasts enhanced macrophage CX3CR1 reporter gene expression (Figure 3a and b). Inhibition of TGF-β1 receptor signaling attenuated this upregulation (Figure 3c). Direct addition of TGF-β significantly increased CX3CR1 reporter gene expression (Figure 3d), also in serum-free media, avoiding effects by TGF-β contained in fetal calf serum (Figure 3e). Similarly, CX3CR1 mRNA expression in wild-type bone marrow was upregulated by TGF-β dose and time dependently (Figure 3f). Human monocytes are subdivided into 3 subpopulations by surface expression of CD14 and CD16,[40] with CX3CR1 expression characteristic of CD14^{+}CD16^{+} cells (Figure 3g). TGF-β significantly enhanced surface CX3CR1 expression on this subtype (Figure 3h).

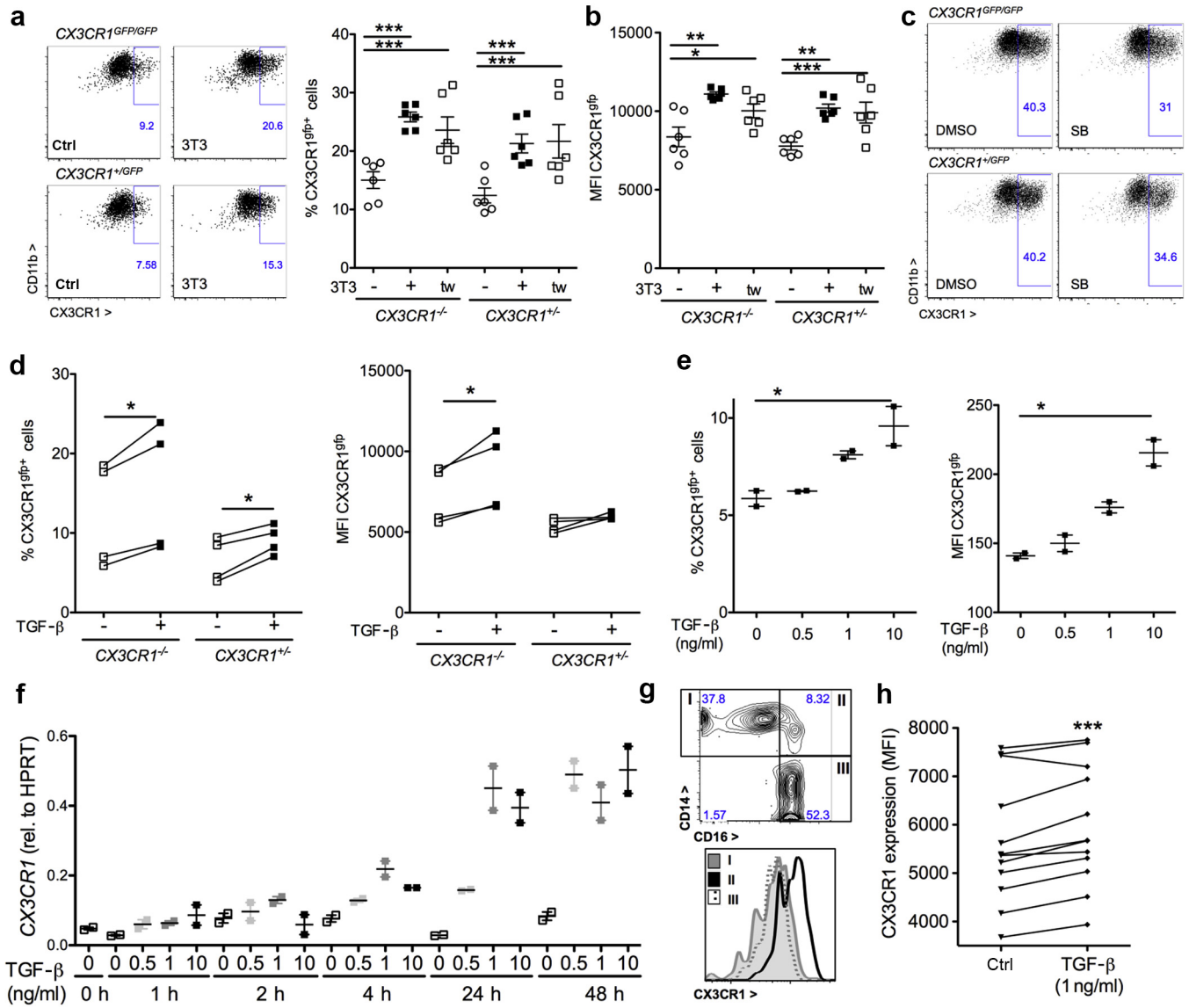

Figure 3 | Fibroblasts and transforming growth factors (TGF)–β induce CX3CR1 expression in murine and human myeloid cells. (**a,b**) *CX3CR1*$^{+/-}$ and *CX3CR1*$^{-/-}$ bone marrow–derived macrophages were co-cultured for 24 hours with 3T3 fibroblasts in direct contact and in transwells (tw). CX3CR1 reporter expression as percentage (**a**) and mean fluorescence intensity (MFI; **b**) ($n = 6$, 3 independent experiments, Dunnett's test after analysis of variance [ANOVA]). (**c**) SMAD3 inhibitor (SB431542, 5 μM) or dimethylsulfoxide (DMSO) control were applied during tw co-cultures of *CX3CR1*$^{+/-}$ and *CX3CR1*$^{-/-}$ bone marrow–derived macrophages with fibroblasts (representative examples of $n = 4$ from 2 independent experiments). (**d**) *CX3CR1*$^{+/-}$ and *CX3CR1*$^{-/-}$ bone marrow–derived macrophages were stimulated with 10 ng/ml TGF-β and percentage and MFI of CX3CR1-driven green fluorescent protein (GFP) quantified ($n = 4$ from 2 independent experiments, paired *t*-tests). (**e**) *CX3CR1*$^{-/-}$ bone marrow macrophages were stimulated with TGF-β in the indicated concentrations for 20 hours in Roswell Park Memorial Institute medium in the absence of fetal calf serum and CX3CR1 reporter expression determined (percentage and MFI, $n = 2$, test for a linear trend after ANOVA). (**f**) CX3CR1 mRNA expression in wild-type bone marrow cells in response to indicated times and concentrations of TGF-β (1 of 2 experiments with parallel results). (**g,h**) Human peripheral blood monocytes were stimulated with 1 ng/ml TGF-β for 2 hours and CX3CR1 surface expression on CD14^{+}CD16^{+} cells (group II in **g**) was assessed by flow cytometry (**h**, $n = 12$ donors from 4 independent experiments, paired *t*-test).

These results show that TGF-β increases murine and human myeloid cell CX3CR1 expression.

Macrophage–mesothelial cell interaction upregulates mesothelial TGF-β and CX3CL1 expression in a CX3CR1-dependent manner

Given the upregulation of macrophage CX3CR1 by TGF-β, mesothelial cell TGF-β production was studied. Sterile PD solution did not alter its expression ($n = 4$, data not shown). However, a combination of IL-1β, TNF-α, and TGF-β significantly enhanced mesothelial cell TGF-β production (Figure 4a).

Macrophages and mesothelial cells are located in close proximity in the peritoneum. Macrophages produced more IL-1β in direct co-culture with mesothelial cells (Figure 4b and c). This outcome depended on direct cellular contact, as

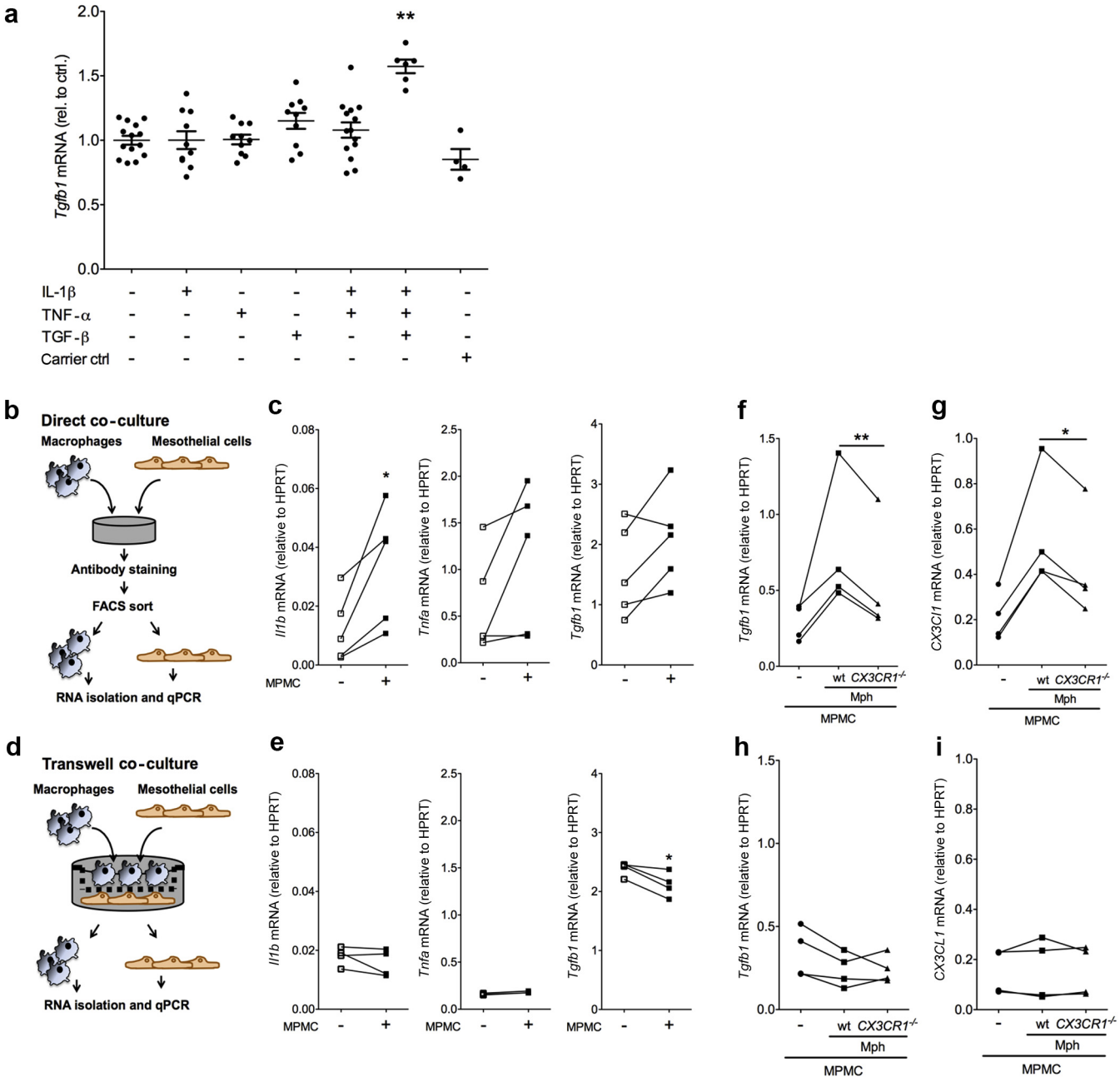

Figure 4 | Cytokines and CX3CR1^{+} macrophages promote mesothelial transforming growth factor (TGF)–β and CX3CL1 expression. (**a**) Murine peritoneal mesothelial cells (MPMC) were stimulated with interleukin (IL)-1β (10 ng/ml), tumor necrosis factor (TNF)–α (10 ng/ml), or TGF-β (10 ng/ml), combinations thereof, or carrier protein control. *Tgfb1* mRNA expression was assessed after 4 hours (n = 6–10 from 3–5 independent experiments, Dunn's tests after analysis of variance). (**b–i**) Direct and transwell co-culture of macrophages and mesothelial cells for assessment of cytokine production. (**b–e**) IL-1β, TNF-α, and TGF-β expression in bone marrow–derived macrophages after 24 hours of direct (**c**) or transwell (**e**) co-culture with MPMC or controls (n = 5 independent experiments, paired t-tests). (**f–i**) MPMCs were incubated directly (**f,g**) or in transwells (**h,i**) with bone marrow–derived macrophages as depicted in (**b**) and (**d**). MPMC TGF-β (**f,h**) and CX3CL1 mRNA expression (**g,i**) was determined by quantitative polymerase chain reaction (qPCR) after cell sorting (n = 4 independent experiments, paired t-tests). FACS, flow cytometry; HPRT, hypoxanthine-guanine phosphoribosyltransferase; Mph, macrophage.

demonstrated by transwell cultures (Figure 4d and e). These results propose macrophage IL-1β as a promoter of mesothelial TGF-β in peritoneal fibrosis. This hypothesis was tested in direct and transwell co-cultures (Figure 4). Direct co-cultures with macrophages induced TGF-β and CX3CL1 mRNA expression in mesothelial cells (Figure 4f and g). However, this increase of TGF-β and CX3CL1 was completely abrogated in transwells (Figure 4h and i), which is consistent with a lack of macrophage IL-1β.

To determine a role of CX3CR1 in this process, wild-type and *CX3CR1*$^{-/-}$ macrophages were compared. Macrophage IL-1β, TNF-α, and TGF-β expression were independent of

CX3CR1 in this setting (Supplementary Figure S11). However, CX3CR1^{+} cells induced significantly more mesothelial TGF-β and CX3CL1 expression in otherwise identical conditions than did control cells from *CX3CR1*$^{-/-}$ mice (Figure 4f,g). These data suggest that macrophage CX3CR1 in direct cellular contact promotes mesothelial cell TGF-β and CX3CL1 expression.

PD fluid upregulates macrophage IL-1β production *in vitro* and *in vivo*

PD solution was investigated as a possible trigger of macrophage cytokine production. It significantly increased IL-1β mRNA expression in both bone marrow–derived and primary peritoneal macrophages (Figure 5a–c), whereas no increase in either TNF-α or TGF-β occurred. Increased secretion of IL-1β protein was detected by enzyme-linked immunosorbent assay (Figure 5d). To test for underlying mechanisms, PD solution components were investigated. A high glucose level increased IL-1β expression and methylglyoxal, a glucose degradation product[41] at a high concentration, tended to increase IL-1β, but not a pH of 5 or lactate (Figure 5e). Also *in vivo,* PD fluid compared with saline solution increased peritoneal IL-1β protein concentration. The increase was significantly stronger in wild-type than in *CX3CR*$^{-/-}$ mice (Figure 5f).

Taken together, these data propose glucose-mediated upregulation of macrophage IL-1β expression as an initial step in PD fluid–induced peritoneal fibrosis.

CX3CL1 is upregulated by inflammatory cytokines in human mesothelial cells and during peritoneal dialysis *in vivo*

To study the situation in human PD, peritoneal cells were recovered from the dialysate. Peritoneal macrophages significantly expressed the CX3CL1 receptor CX3CR1 in human PD (Figure 6a). In parallel to the murine situation (Figure 5b and c), restimulation with PD fluid significantly increased IL-1β and also TNF-α expression in human peritoneal macrophages (Figure 6b). The CX3CR1 ligand CX3CL1 was detected on human peritoneal mesothelium identified by podoplanin expression (Figure 6c).[42] CX3CL1 regulation was studied further in primary human peritoneal mesothelial cells from healthy donors. After stimulation with IL-1β, TNF-α, and TGF-β, CX3CL1 was significantly upregulated, with a most marked effect of IL-1β (Figure 6d), which peaked at 4 hours (Supplementary Figure S12). Histologic staining of human peritoneal membrane sections revealed marked mesothelial CX3CL1 expression in peritoneal dialysis, while it was hardly visible on healthy, unstimulated peritoneum (Figure 6e). Although, similar to the mouse, soluble CX3CL1 within the peritoneal cavity was below the detection limit (data not shown), serum CX3CL1 levels were significantly elevated in patients who recently had begun PD compared with patients who had chronic kidney disease before start of renal replacement therapy and long-term stable patients undergoing PD (Figure 6f, clinical characteristics in Supplementary Table S2).

The impact of these factors on profibrotic mediator expression in human peritoneal mesothelium was tested next. IL-1β, TNF-α and TGF-β together moderately promoted TGF-β expression (Figure 6g). Direct co-culture with human monocytes effectively promoted TGF-β along with extracellular matrix collagen and fibronectin expression in human peritoneal mesothelium (Figure 6h).

These results showing human peritoneal CX3CR1 and CX3CL1 expression in PD *in vivo* and similar regulatory mechanisms as in the murine model *in vitro* suggest that CX3CL1–CX3CR1 interaction is relevant for patients undergoing PD.

DISCUSSION

Our experiments show that CX3CR1–CX3CL1 interaction promotes peritoneal fibrosis. The data propose that in macrophage–mesothelial cell contact, macrophage CX3CR1 and IL-1β enhance mesothelial CX3CL1. In addition, CX3CR1 increases mesothelial TGF-β production that in turn promotes CX3CR1 on myeloid cells, closing a profibrotic loop.

CX3CL1 (fractalkine) was upregulated by stimulation in murine and human peritoneal mesothelium, and increased CX3CL1 concentrations were detected in sera of patients undergoing PD. IL-1β was instrumental in CX3CL1 upregulation. Macrophage IL-1β production was enhanced by direct contact with mesothelial cells. In addition, it was induced in bone marrow–derived and primary peritoneal macrophages by PD solution. In more aggressive peritoneal injury models, such as bacterial peritonitis[16] or injection of staphylococcal lysates,[17] exogenous substances induce high amounts of IL-1β that promote acute fibrosis—for example, peritoneal fibrosis after exposure to gadolinium-based agents[43] and pulmonary fibrosis.[44] The role of CX3CR1 could be further investigated in such highly inflammatory models that lead to major mesothelial thickening and ultrafiltration loss; however, caution is necessary because disease mechanisms may differ from PD. Still, the moderate macrophage accumulation in the peritoneal cavity and wall observed with PD[11] already amplifies the likelihood of macrophage–mesothelial interaction. Our data illustrate the impact of interactions of macrophages and mesothelial cells on the TGF-β–mediated remodeling[3-6] in peritoneal fibrosis.

CX3CL1 exists as a stalked and as a soluble cytokine. Our data show that cellular contact with CX3CR1-bearing macrophages significantly promotes mesothelial expression of TGF-β and CX3CL1. We and other investigators[25] found similar expression of many other cytokines in CX3CR1^{+} and CX3CR1-deficient macrophages. Our transwell data suggest induction of close leukocyte–mesothelial contact or even a direct signaling pathway of stalked CX3CL1 as the underlying mechanism. Signaling of stalked CX3CL1 remains to be delineated in future studies and also may be relevant in other cell types.[45] In addition to the chronic peritoneal fibrosis reported here, high mesothelial CX3CL1 has been found in human peritoneal endometriosis and peritoneal metastasis.[35,36] Its functional role in these types of

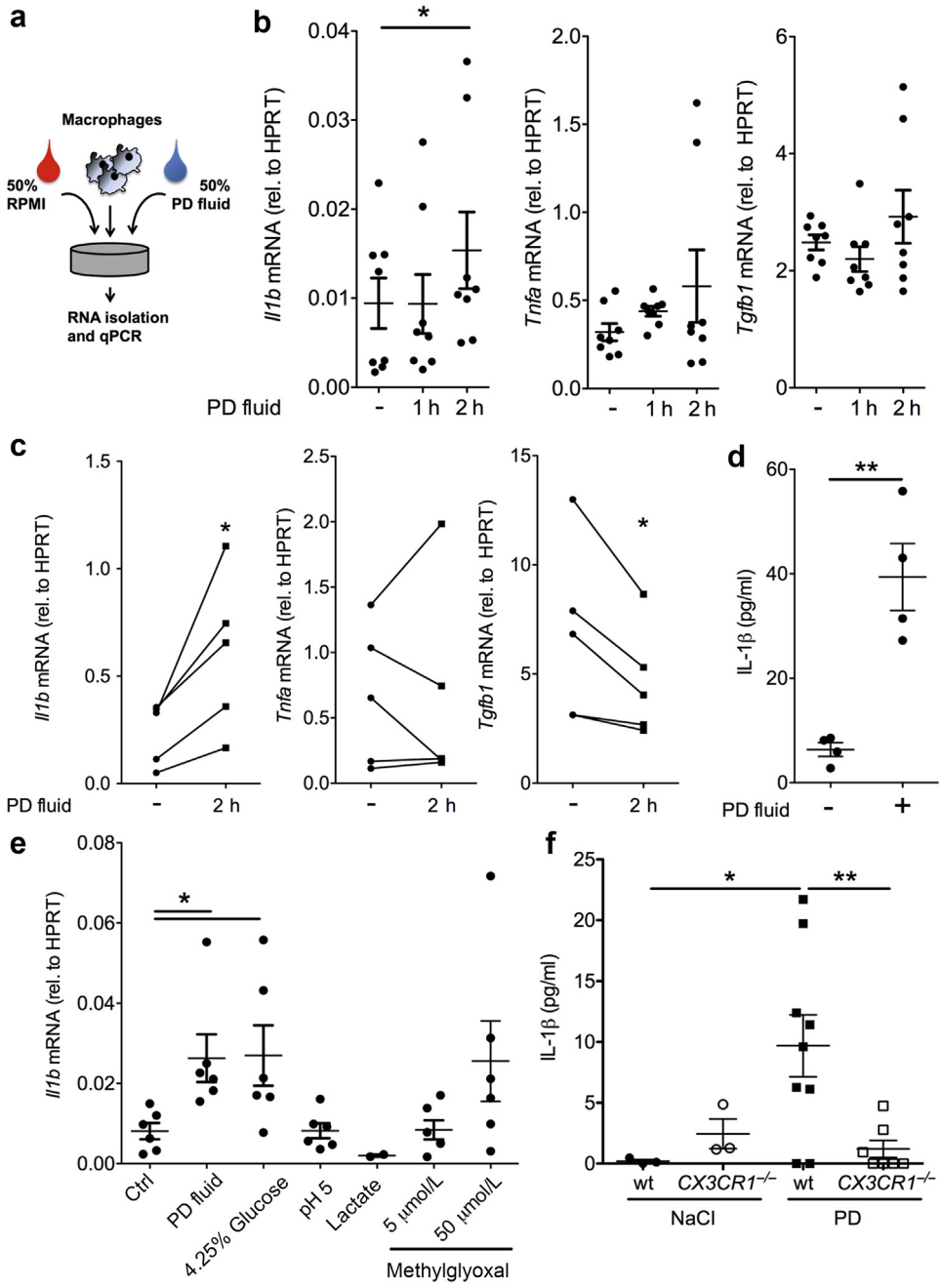

Figure 5 | Peritoneal dialysis (PD) solution enhances macrophage interleukin (IL)-1β mRNA and protein production. (**a–c**) PD fluid (50%) was added to macrophage cultures. Bone marrow–derived (**b**) and primary peritoneal (**c**) macrophage culture in full Roswell Park Memorial Institute medium (RPMI) for 1 to 2 hours and IL-1β, tumor necrosis factor–α, and transforming growth factor–β expression determined by quantitative polymerase chain reaction (qPCR) (**b**, $n = 8$, 4 independent experiments, Dunnett's tests after analysis of variance [ANOVA]; **c**, $n = 5$ independent experiments, paired *t*-tests). (**d**) IL-1β secretion into the supernatant of primary peritoneal macrophages was assessed 24 hours after 2 hours of incubation with PD fluid ($n = 4$ independent experiments, *t*-test). (**e**) IL-1β mRNA expression after 2 hours of incubation with 50% PD fluid, glucose, pH 5.0, lactate, and methylglyoxal at the indicated concentrations ($n = 6$, Dunn's test after ANOVA). (**f**) IL-1β concentration in the peritoneal lavage after 6-week PD or control saline (NaCl) treatment in wild-type and *CX3CR1*$^{-/-}$ mice ($n = 3$ NaCl, 7–9 PD mice per genotype, Bonferroni after ANOVA). HPRT, hypoxanthine-guanine phosphoribosyltransferase.

peritoneal disease remains to be defined, but our data also provide possible regulatory mechanisms for these findings.

Our results show that TGF-β promotes macrophage CX3CR1 expression, which extends previous observations in other cell types[29–32] and suggests TGF-β as a mediator of macrophage CX3CR1 upregulation in other types of fibrosis such as scleroderma[46] and wound healing.[22] Interestingly, a very recent report mentions high expression of CX3CR1 in PD effluents detected by gene expression array.[10] This finding complements our results in human patients in a non–

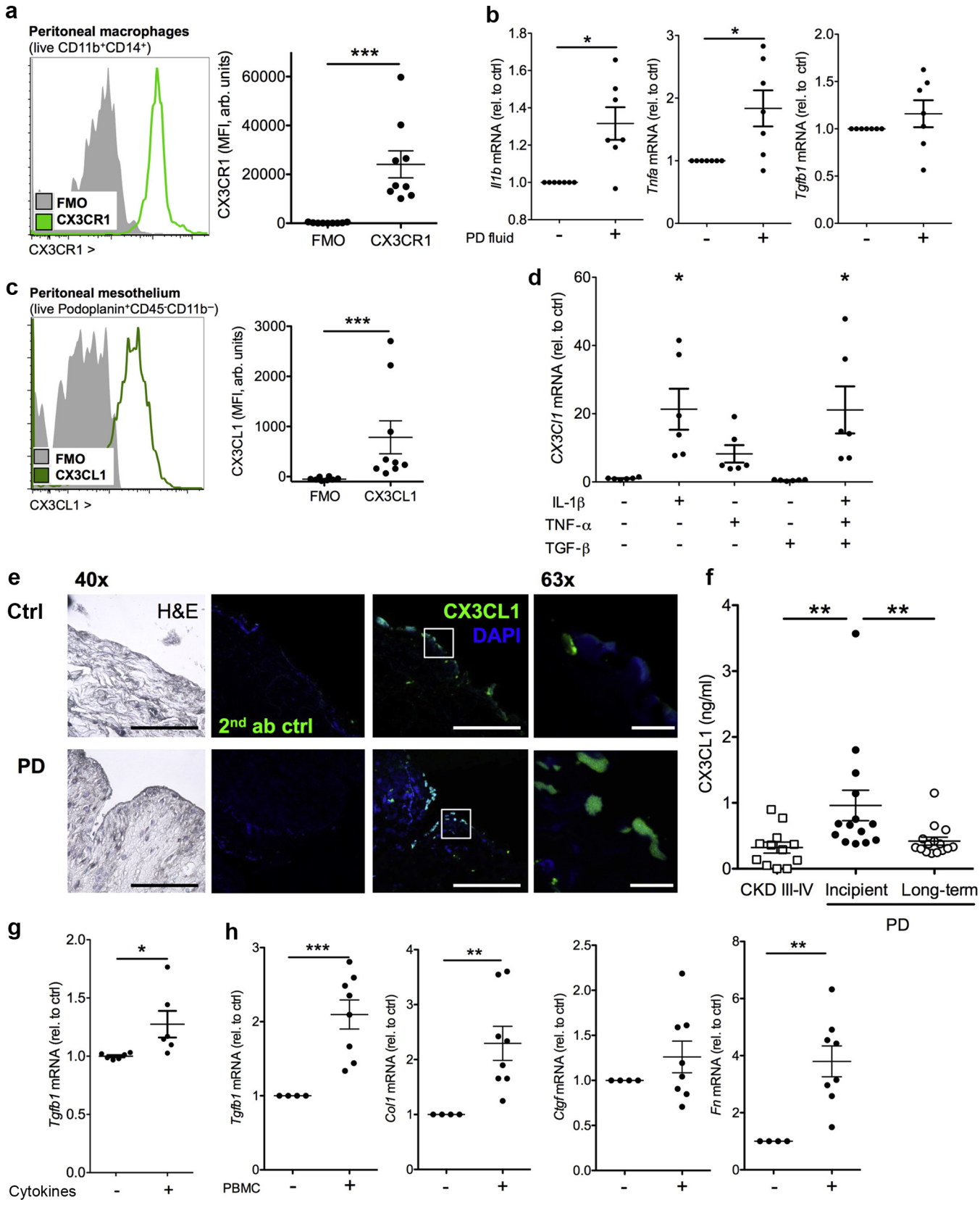

Figure 6 | CX3CL1 regulation in human peritoneal mesothelial cells and peritoneal dialysis (PD) *in vivo*. (**a**,**b**) Human peritoneal macrophages were recovered from effluent of patients undergoing PD. (**a**) Live $CD11b^{+}CD14^{+}$ macrophages were studied for CX3CR1 expression (example and analysis of $n = 9$ donors, Mann-Whitney test). (**b**) Adhesion-enriched peritoneal macrophages were restimulated with PD fluid after an overnight rest and interleukin (IL)-1β, tumor necrosis factor (TNF)–α, and transforming growth factor (TGF)–β expression was (continued)

hypothesis-driven approach, while our experiments provide an underlying mechanism of CX3CR1 upregulation and a rationale for therapeutic implications of their findings.

CX3CR1 promoted fibrosis in our model of chronic exposure to PD solution despite limited peritoneal fibrosis development in the C57BL/6J background.[38] Mechanistically, CX3CR1 enhanced mesothelial cell TGF-β production in response to macrophages *in vitro*. This finding parallels findings on the CX3CR1 role in fibrosis of others organs[21–23] and decreased renal TGF-β with pharmacologic CX3CR1 inhibition.[47] However, antifibrotic CX3CR1 effects also have been reported.[24–26] Underlying mechanisms that result in diverging effects may include local expression levels of CX3CL1, which was high in the peritoneal mesothelium, sex differences in receptor regulation, with more stable signaling in females,[48] and the time course of ligand and receptor expression, as observed in muscle necrosis.[27] For therapeutic application, low molecular weight CX3CR1 blockers have been published,[49] but commencement of interventional studies has not yet been reported (clinicaltrials.gov, accessed 18 June 2018). Given the presence of CX3CR1 in many cell systems and the need for continuous application,[50] extensive safety testing is mandatory. However, integration into peritoneal dialysate solutions in a nonabsorbable formulation would confer localized action of a therapeutic anti-CX3CR1 agent.

In summary, our experiments show that CX3CR1 mediates a profibrotic loop in chronic peritoneal fibrosis *in vivo* by promoting direct mesothelial activation. CX3CR1 should be tested further as a new target for preservation of peritoneal function in persons undergoing PD.

METHODS

Animals

Wild-type C57BL/6, *CX3CR1*$^{-/-}$ (=*CX3CR1*$^{GFP/GFP}$) and heterozygous *CX3CR1*$^{+/-}$ mice (all CD45.2 on C57BL/6J background) and congenic B6.SJL-*Ptprc*^{a}*Pepc*^{b}/BoyJ (CD45.1) mice (The Jackson Laboratory, Bar Harbor, ME) genotyped by polymerase chain reaction were kept in specific-pathogen–free conditions and used in age- and sex-matched groups. Animal experiments were approved by the Landesamt für Verbraucherschutz und Lebensmittelsicherheit, Lower Saxony, Germany (2082,2117). Full blood counts were measured in an automated analyzer (vet abc, Scil Animal Care Company, Viernheim, Germany). Surgical procedures and organ harvest are described in the online supplement.

Murine cell culture and stimulation

A murine mesothelial cell line was generated previously in our laboratory and cultured as described.[11] For assessment of CX3CL1 expression, the murine mesothelial cell line, mouse cardiac endothelial cells (CELLutions Biosystems Inc, Toronto, Ontario, Canada), or 3T3 murine fibroblasts (ACC59, DSMZ GmbH, Braunschweig, Germany) were stimulated for 4 hours with 10 ng/ml TNF-α, IL-1β, interferon-γ (PeproTech GmbH, Hamburg, Germany), and/or TGF-β (R&D Systems, Minneapolis, MN) as indicated.

For macrophage culture, bone marrow cells enriched by adhesion to plastic for 6 hours were cultured for 7 days in full Roswell Park Memorial Institute medium. Murine peritoneal macrophages were recovered by flushing the peritoneal cavity with sterile phosphate-buffered saline solution and enriched by 6-h adhesion to cell culture dishes and subsequent washing. Twenty-four hour TGF-β stimulation (10 ng/ml, BioLegend, San Diego, CA) and cell survival (Roswell Park Memorial Institute medium without fetal calf serum) with TGF-β (10 ng/ml) and/or CX3CL1 (20 nM PeproTech, Rocky Hill, NJ) as indicated was assessed on day 7. Cell proliferation was measured by CellTracker Orange CMTMR (Thermo Fisher Scientific, Dreieich, Germany, 10 μM) dye dilution. Sterile 4.25% glucose lactate-buffered PD dialysis solution (CAPD/DPCA3, stay-safe, Fresenius Medical Care, Bad Homburg, Germany) or glucose (42.5 g/l), sodium lactate (3.925 g/l), methylglyoxal (5 and 50 μmol/l) in sterile phosphate-buffered saline solution or sterile phosphate-buffered saline solution titrated to a pH of 5.0 using hydrochloric acid (all from Sigma-Aldrich Chemie GmbH, Munich, Germany) were added as indicated.

For direct co-culture or transwell experiments with macrophages, murine mesothelial cell line or 3T3 cells were plated into wells or transwells (BRANDplates Insert Strips, PET membrane 0.4 μm, Brand GmbH + CO KG, Wertheim, Germany). Co-culture with the murine mesothelial cell line was in full Roswell Park Memorial Institute medium, with 3T3 in full Dulbecco's modified Eagle's medium with 15 mmol/l *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid. Bone marrow–derived macrophages were added at a 1:1 ratio. SMAD3 inhibitor (SB431542, 5 μM) and dimethyl sulfoxide controls were used as indicated. For quantitative polymerase chain reaction analysis after macrophage–mesothelial cell direct co-culture, co-cultured and control cells were sorted (FACSAria II, Becton Dickinson, Franklin Lakes, NJ) into live CD11b^{+} and CD11b^{-} populations.

Figure 6 | (continued) measured after 2 hours ($n = 7$ donors in independent experiments, Wilcoxon signed rank test). (**c**) Peritoneal mesothelial cells in PD effluents were identified as live Podoplanin^{+}CD45^{-}CD11b^{-} cells and CX3CL1 expression was assessed by flow cytometry (example and analysis of $n = 9$ donors, Mann-Whitney test). (**d**) CX3CL1 expression in cultured human primary peritoneal mesothelial cells was determined by quantitative polymerase chain reaction after stimulation with recombinant IL-1β (10 ng/ml), TNF-α (10 ng/ml), or TGF-β1 (10 ng/ml), all combined or carrier control after 4 hours ($n = 6$ from 3 experiments, Dunn's tests after analysis of variance). (**e**) Peritoneal mesothelium from a healthy control subject and a patient undergoing PD was stained for CX3CL1 (green, 4′,6-diamidino-2-phenylindole [DAPI] nuclear counterstain [blue], original magnification ×40 and ×63, bars = 100 μm and 10 μm, typical examples). (**f**) Serum CX3CL1 was determined in patients with non–dialysis dependent chronic kidney disease (CKDIII-IV) and recent and stable long-term PD patients ($n = 12$–15, Dunn's multiple comparison test). (**g**) TGF-β mRNA expression was assessed in human primary peritoneal mesothelial cells after 4 hours of stimulation with recombinant IL-1β (10 ng/ml), TNF-α (10 ng/ml), and TGF-β1 ($n = 6$ from 3 experiments, Wilcoxon signed rank test). (**h**) TGF-β, collagen 1, connective tissue growth factor (CTGF), and fibronectin (Fn) mRNA was measured after co-culture with peripheral blood mononuclear cells (PBMCs; $n = 8$ PBMC donors in 4 independent experiments, Wilcoxon signed rank tests). ab, antibody; arb, arbitrary; FMO, fluorescence minus one; H&E, hematoxylin and eosin; MFI, mean fluorescence intensity. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

Human peripheral blood mononuclear cells and mesothelial cell culture

Human peripheral blood mononuclear cells from healthy blood donors, sera for CX3CL1 assessment from patients with chronic kidney disease and those undergoing PD and peritoneal dialysate, and membrane samples were obtained after written informed consent according to the declaration of Helsinki and local ethics board approval (Hanover Medical School 2010/807, 2014/6617). Blood mononuclear cells were isolated by density gradient centrifugation (Biocoll 1.077, Biochrom, Berlin, Germany) and stimulated with recombinant human TGF-β (1 ng/ml) for 2 hours in Roswell Park Memorial Institute medium as indicated. Peritoneal fluid cells were concentrated by centrifugation before analysis by flow cytometry. Adhesion-enriched peritoneal macrophages were rested overnight before restimulation with PD fluid for 2 hours.

Primary human peritoneal mesothelial cells were isolated as described[11] and used in passages 2–5. They were stimulated for 4 hours with 10 ng/ml recombinant human TNF-α, IL-1β (PeproTech), and/or TGF-β (BioLegend) as indicated. Co-culture with peripheral blood mononuclear cells (24 hours, ratio 1:1) and sorting protocols were as for murine cells.

Enzyme-linked immunosorbent assay

Mouse IL-1β and mouse and human CX3CL1 measurements were performed in duplicates using Quantikine enzyme-linked immunosorbent assay (R&D Systems) according to the manufacturer's instructions. Murine peritoneal lavage was obtained in PD solution from *CX3CR1*$^{-/-}$ mice undergoing PD and controls (2.4 ml average volume, not different between groups) because soluble CX3CL1 was below the detection limit in almost all wild-type mice independent of therapy (data not shown), possibly indicating receptor binding.[39] For assessment of CX3CL1 concentration in cell culture supernatants, cells were washed with phosphate-buffered saline solution after 4 hours of stimulation with the indicated cytokines and incubated for 24 hours in fresh medium.

RNA isolation, gene array, and real-time polymerase chain reaction

RNA was isolated using NucleoSpin RNA II Kit (Macherey-Nagel GmbH & Co. KG, Duren, Germany) and reversely transcribed with M-MLV-RT (Promega GmbH, Mannheim, Germany) according to the manufacturer's instructions. Real-time polymerase chain reaction was performed in duplicates on a LightCycler 480 or LightCycler 96 using SYBR Green (Roche, Grenzach-Wyhlen, Germany). Primer sequences were selected using PrimerBank and are reported in the Supplementary Methods. Products were confirmed by melting curve analysis and gel electrophoresis. Data were analyzed with hypoxanthine guanine phosphoribosyl transferase (*HPRT*) as a reference gene using LinRegPCR software.

Enzymatic digestion of tissues and flow cytometry

Preparation and enzymatic digestion of tissues, antibodies, and other staining reagents are reported in the Supplementary Methods. Flow cytometry analysis was performed on a Becton-Dickinson FACS Canto or LSR II (Becton, Dickinson and Company, Franklin Lakes, NJ). Data were analyzed using FlowJo software (Tree Star Inc., Ashland, OR).

Statistical Analysis

A normal distribution was assumed for continuous biological variables because most follow a Gaussian distribution. A 2-tailed Student *t*-test was used to compare 2 conditions; the Mann-Whitney test was used in case of non-normal distribution by the Kolmogorov-Smirnov test. If more than 2 conditions were compared, Bonferroni's test of selected conditions or test of linear trend was applied after analysis of variance, and, in case of normalization of baseline values, Wilcoxon tests and nonparametric analyses of variance were used as indicated in the figure legends. P values <0.05 were considered significant. Data are expressed as mean ± SEM. P values are indicated as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary Methods.

Table S1. Characteristics of wild-type and CX3CR1$^{-/-}$ mice after 6 weeks of peritoneal dialysis.

Table S2. Characteristics of patients with chronic kidney disease and patients undergoing peritoneal dialysis.

Figure S1. Peritoneal dialysis (PD) fluid does not affect CX3CL1 expression in the mesothelium. Mouse peritoneal mesothelial cells were stimulated with a combination of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and transforming growth factor (TGF)-β or left as controls in the presence or absence of 25% PD fluid supplementation to the growth media for 4 hours. CX3CL1 mRNA was assessed by quantitative polymerase chain reaction ($n = 3$ independent experiments, Bonferroni after analysis of variance).

Figure S2. CX3CR1 expressing leukocytes in the peritoneal wall**.** Peritoneal wall leukocytes expressing CX3CR1 reporter green fluorescent protein were assessed for myeloid cell marker CD11b, T-cell marker CD3, natural killer cell marker NK 1.1, and B-cell marker CD19 in resting mice and 16 hours and 4 days (d4) after injection of peritoneal dialysis (PD) solution ($n = 5$–6 per time point from 2 independent experiments).

Figure S3. No evidence of enhanced apoptosis in the absence of CX3CR1 with and without transforming growth factor (TGF)-β and fractalkine (CX3CL1) exposition *in vitro*. (**A,B**) Apoptosis of bone marrow–derived *CX3CR1*$^{+/-}$ (**A**) and *CX3CR1*$^{-/-}$ (**B**) macrophages was assessed by annexin V staining after 24 hours of serum starvation with and without recombinant TGF-β (10 ng/ml) and fractalkine (20 nM) as indicated (gated for CD11b^{+} live cells, $n = 6$ from 3 independent experiments, one-way analysis of variance).

Figure S4. Bone marrow stem cell differentiation in CX3CR1$^{+/-}$ and CX3CR1$^{-/-}$ mice after 6 weeks of peritoneal dialysis (PD) treatment. (**A–E)** Hematopoietic progenitors were gated as indicated for hematopoietic stem cells (HSCs, **B**), myeloid progenitors (MPs, **C**), common myeloid progenitors (CMPs, **D**) and granulocyte macrophage progenitors (GMPs, **E**) in bone marrow. **(B–E)** Counts were assessed in control mice and mice after 6 weeks of PD ($n = 7$–9 per group from 2 independent experiments, Bonferroni after one-way analysis of variance).

Figure S5. Bone-marrow-derived CX3CR1$^{+/-}$ and CX3CR1$^{-/-}$ macrophage proliferation in the presence and absence of transforming

growth factor (TGF)-β and fractalkine *in vitro*. Bone marrow mononuclear cells were stained with CellTracker Orange (Thermo Fisher Scientific, Dreieich, Germany), and proliferation was assessed by measurement of marker dilution after 6 days of being cultured in the presence or absence of recombinant fractalkine (20 nM) and TGF-β (10 ng/ml) and as indicated ($n = 6$ from 3 independent experiments, *t*-tests).

Figure S6. Monocyte proliferation after peritoneal dialysis and in resting CX3CR1$^{+/-}$ and CX3CR1$^{-/-}$ mice. (**A–F**) Ki67 proliferation marker expression was assessed in *CX3CR1*$^{-/-}$ and *CX3CR1*$^{+/-}$ mice after 6 weeks of peritoneal dialysis (**A–C**) and at rest (**D–F**) in bone marrow (**A,D**), peripheral blood (**B,E**), peritoneal wall (**C,F**) monocytes (**A,B,D,E**) and myeloid phagocytes (**C,F**) and their subtypes as indicated ($n = 7$–8 per group from 2 independent experiments, *t*-tests).

Figure S7. Peritoneal wall leukocytes after 6 weeks' exposure to peritoneal dialysis fluid. **(A–E)** Peritoneal wall leukocytes were assessed by flow cytometry after enzymatic tissue digestion in *CX3CR1*$^{-/-}$ and wild-type mice after 6 weeks of peritoneal dialysis (PD) compared with untreated controls. Gating for live cells and subgroups was performed as depicted in (**A**) using the spleen as positive control for lymphocyte stainings. Counts of all live CD45^{+} leukocytes (**B**), CD11b^{+} myeloid cells (**C**), αβTCR^{+} T cells (**D**), and CD19^{+} B cells (**E**) did not differ significantly between the groups ($n = 10$–14/PD group, 3 independent experiments, analysis of variance).

Figure S8. Peritoneal wall and lavage leukocytes after short-term exposure to peritoneal dialysis (PD) fluid. Peritoneal wall (**A–D**) and peritoneal lavage (**E–G**) leukocytes were assessed for myeloid cell marker CD11b, T-cell marker CD3, natural killer cell marker NK 1.1, and B-cell marker CD19 in resting mice and 16 hours and 4 days (d4) after injection of PD solution ($n = 5$–6 per time point from 2 independent experiments).

Figure S9. Peripheral blood monocyte counts during peritoneal dialysis (PD) treatment. (**A**) Blood monocytes in mice defined by CD115 expression were subdivided in Gr1^{high}CD11c^{low}CX3CR1^{low} and Gr1^{low}CD11c^{high}CX3CR1^{high} cells. This is aided by green fluorescent protein (GFP) reporter gene expression under the CX3CR1 promoter with different signal intensity in *CX3CR1*$^{-/-}$ and *CX3CR1*$^{+/-}$ mice (examples). (**B–F**) *CX3CR1*$^{-/-}$ and wild-type mice were treated with peritoneal solution daily for 6 weeks and blood monocytes were assessed by an automated analyzer and flow cytometry.
Total monocyte counts (**B**), relative (**C,D**) and absolute (**E,F**) Gr1^{high}CD11c^{low}CX3CR1^{low} (**C,E**) and Gr1^{low}CD11c^{high}CX3CR1^{high} (**D,F**) concentrations are shown ($n = 14$–15 per group, 2-way analysis of variance results for genotype).

Figure S10. Effect of peritoneal dialysis (PD) fluid on macrophage CX3CR1 expression. Mouse bone marrow–derived (**A**) and primary peritoneal (**B**) macrophages were exposed to PD fluid (50%, 50% full Roswell Park Memorial Institute medium) for the indicated periods. CX3CR1 expression was studied by quantitative polymerase chain reaction (**A**, $n = 6$, 4 independent experiments, Dunnett's after analysis of variance; **B**, $n = 5$ independent experiments, *t*-test).

Figure S11. Macrophage cytokine expression in response to co-culture with mesothelial cells. (**A–C**) Interleukin (IL)-1β (**A**), tumor necrosis factor (TNF)-α (**B**) and transforming growth factor (TGF)-β (**C**) cytokine expression in wild-type (wt) and *CX3CR1*$^{-/-}$ macrophages was determined after 24 hours of co-culture with peritoneal mesothelial cells ($n = 4$–5 independent experiments, paired *t*-tests).

Figure S12. Time course of human peritoneal mesothelial cell CX3CL1 mRNA expression in response to stimulation with interleukin (IL)-1β. Human primary peritoneal mesothelial cells were stimulated with recombinant IL-1β (10 ng/ml) for the indicated periods and CX3CL1 mRNA expression level was determined by quantitative polymerase chain reaction ($n = 4$, Dunn's after analysis of variance).

Supplementary material is linked to the online version of the paper at www.kidney-international.org.

REFERENCES

1. Jain AK, Blake P, Cordy P, et al. Global trends in rates of peritoneal dialysis. *J Am Soc Nephrol*. 2012;23:533–544.
2. Li PK, Chow KM, Van de Luijtgaarden MW, et al. Changes in the worldwide epidemiology of peritoneal dialysis. *Nat Rev Nephrol*. 2017;13:90–103.
3. Davies SJ. Peritoneal solute transport and inflammation. *Am J Kidney Dis*. 2014;64:978–986.
4. Bajo MA, Del Peso G, Teitelbaum I. Peritoneal membrane preservation. *Semin Nephrol*. 2017;37:77–92.
5. Zhou Q, Bajo MA, Del Peso G, et al. Preventing peritoneal membrane fibrosis in peritoneal dialysis patients. *Kidney Int*. 2016;90:515–524.
6. Morelle J, Devuyst O. Water and solute transport across the peritoneal membrane. *Curr Opin Nephrol Hypertens*. 2015;24:434–443.
7. Schaefer B, Bartosova M, Macher-Goeppinger S, et al. Quantitative histomorphometry of the healthy peritoneum. *Sci Rep*. 2016;6:21344.
8. Williams JD, Craig KJ, Topley N, et al. Morphologic changes in the peritoneal membrane of patients with renal disease. *J Am Soc Nephrol*. 2002;13:470–479.
9. Lai KN, Lai KB, Lam CW, et al. Changes of cytokine profiles during peritonitis in patients on continuous ambulatory peritoneal dialysis. *Am J Kidney Dis*. 2000;35:644–652.
10. Liao CT, Andrews R, Wallace LE, et al. Peritoneal macrophage heterogeneity is associated with different peritoneal dialysis outcomes. *Kidney Int*. 2017;91:1088–1103.
11. Wang L, Balzer MS, Rong S, et al. Protein kinase C alpha inhibition prevents peritoneal damage in a mouse model of chronic peritoneal exposure to high-glucose dialysate. *Kidney Int*. 2016;89:1253–1267.
12. Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. *Immunity*. 2016;44:450–462.
13. Bellon T, Martinez V, Lucendo B, et al. Alternative activation of macrophages in human peritoneum: implications for peritoneal fibrosis. *Nephrol Dial Transplant*. 2011;26:2995–3005.
14. Wang J, Jiang ZP, Su N, et al. The role of peritoneal alternatively activated macrophages in the process of peritoneal fibrosis related to peritoneal dialysis. *Int J Mol Sci*. 2013;14:10369–10382.
15. Kalble F, Damaske J, Heide D, et al. Selective blocking of TNF receptor 1 attenuates peritoneal dialysis fluid induced inflammation of the peritoneum in mice. *PloS One*. 2016;11:e0163314.
16. Hautem N, Morelle J, Sow A, et al. The NLRP3 inflammasome has a critical role in peritoneal dialysis-related peritonitis. *J Am Soc Nephrol*. 2017;28:2038–2052.
17. Fielding CA, Jones GW, McLoughlin RM, et al. Interleukin-6 signaling drives fibrosis in unresolved inflammation. *Immunity*. 2014;40:40–50.
18. Thomas G, Tacke R, Hedrick CC, et al. Nonclassical patrolling monocyte function in the vasculature. *Arterioscler Thromb Vasc Biol*. 2015;35:1306–1316.
19. White GE, Greaves DR. Fractalkine: a survivor's guide: chemokines as antiapoptotic mediators. *Arterioscler Thromb Vasc Biol*. 2012;32:589–594.
20. Kassianos AJ, Wang X, Sampangi S, et al. Fractalkine-CX3CR1-dependent recruitment and retention of human CD1c+ myeloid dendritic cells by in vitro-activated proximal tubular epithelial cells. *Kidney Int*. 2015;87:1153–1163.
21. Furuichi K, Gao JL, Murphy PM. Chemokine receptor CX3CR1 regulates renal interstitial fibrosis after ischemia-reperfusion injury. *Am J Pathol*. 2006;169:372–387.
22. Ishida Y, Gao JL, Murphy PM. Chemokine receptor CX3CR1 mediates skin wound healing by promoting macrophage and fibroblast accumulation and function. *J Immunol*. 2008;180:569–579.
23. Arai M, Ikawa Y, Chujo S, et al. Chemokine receptors CCR2 and CX3CR1 regulate skin fibrosis in the mouse model of cytokine-induced systemic sclerosis. *J Dermatol Sci*. 2013;69:250–258.
24. Peng X, Zhang J, Xiao Z, et al. CX3CL1-CX3CR1 Interaction increases the population of Ly6C(-)CX3CR1(hi) macrophages contributing to unilateral ureteral obstruction-induced fibrosis. *J Immunol*. 2015;195:2797–2805.
25. Engel DR, Krause TA, Snelgrove SL, et al. CX3CR1 reduces kidney fibrosis by inhibiting local proliferation of profibrotic macrophages. *J Immunol*. 2015;194:1628–1638.

26. Karlmark KR, Zimmermann HW, Roderburg C, et al. The fractalkine receptor CX(3)CR1 protects against liver fibrosis by controlling differentiation and survival of infiltrating hepatic monocytes. *Hepatology*. 2010;52:1769–1782.
27. Arnold L, Henry A, Poron F, et al. Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. *J Exp Med*. 2007;204:1057–1069.
28. Morimura S, Oka T, Sugaya M, et al. CX3CR1 deficiency attenuates imiquimod-induced psoriasis-like skin inflammation with decreased M1 macrophages. *J Dermatol Sci*. 2016;82:175–188.
29. Dong L, Nordlohne J, Ge S, et al. T cell CX3CR1 mediates excess atherosclerotic inflammation in renal impairment. *J Am Soc Nephrol*. 2016;27:1753–1764.
30. Abutbul S, Shapiro J, Szaingurten-Solodkin I, et al. TGF-beta signaling through SMAD2/3 induces the quiescent microglial phenotype within the CNS environment. *Glia*. 2012;60:1160–1171.
31. Chen S, Luo D, Streit WJ, et al. TGF-beta1 upregulates CX3CR1 expression and inhibits fractalkine-stimulated signaling in rat microglia. *J Neuroimmunol*. 2002;133:46–55.
32. Song KH, Park J, Park JH, et al. Fractalkine and its receptor mediate extracellular matrix accumulation in diabetic nephropathy in mice. *Diabetologia*. 2013;56:1661–1669.
33. Garcia GE, Xia Y, Chen S, et al. NF-kappaB-dependent fractalkine induction in rat aortic endothelial cells stimulated by IL-1beta, TNF-alpha, and LPS. *J Leukoc Biol*. 2000;67:577–584.
34. Matsumiya T, Ota K, Imaizumi T, et al. Characterization of synergistic induction of CX3CL1/fractalkine by TNF-alpha and IFN-gamma in vascular endothelial cells: an essential role for TNF-alpha in post-transcriptional regulation of CX3CL1. *J Immunol*. 2010;184:4205–4214.
35. Gurler Main H, Xie J, Muralidhar GG, et al. Emergent role of the fractalkine axis in dissemination of peritoneal metastasis from epithelial ovarian carcinoma. *Oncogene*. 2017;36:3025–3036.
36. Shimoya K, Zhang Q, Temma-Asano K, et al. Fractalkine in the peritoneal fluid of women with endometriosis. *Int J Gynaecol Obstet*. 2005;91:36–41.
37. Ishida Y, Hayashi T, Goto T, et al. Essential involvement of CX3CR1-mediated signals in the bactericidal host defense during septic peritonitis. *J Immunol*. 2008;181:4208–4218.
38. Walkin L, Herrick SE, Summers A, et al. The role of mouse strain differences in the susceptibility to fibrosis: a systematic review. *Fibrogenesis Tissue Repair*. 2013;6:18.
39. Shah R, O'Neill SM, Hinkle C, et al. Metabolic effects of CX3CR1 deficiency in diet-induced obese mice. *PloS One*. 2015;10:e0138317.
40. Ziegler-Heitbrock L, Ancuta P, Crowe S, et al. Nomenclature of monocytes and dendritic cells in blood. *Blood*. 2010;116:e74–e80.
41. Zeier M, Schwenger V, Deppisch R, et al. Glucose degradation products in PD fluids: do they disappear from the peritoneal cavity and enter the systemic circulation? *Kidney Int*. 2003;63:298–305.
42. Schacht V, Dadras SS, Johnson LA, et al. Up-regulation of the lymphatic marker podoplanin, a mucin-type transmembrane glycoprotein, in human squamous cell carcinomas and germ cell tumors. *Am J Pathol*. 2005;166:913–921.
43. Schmidt-Lauber C, Bossaller L, Abujudeh HH, et al. Gadolinium-based compounds induce NLRP3-dependent IL-1beta production and peritoneal inflammation. *Ann Rheum Dis*. 2015;74:2062–2069.
44. Cassel SL, Eisenbarth SC, Iyer SS, et al. The Nalp3 inflammasome is essential for the development of silicosis. *Proc Natl Acad Sci U S A*. 2008;105:9035–9040.
45. Ludwig A, Weber C. Transmembrane chemokines: versatile 'special agents' in vascular inflammation. *Thrombosis Haemost*. 2007;97:694–703.
46. Hasegawa M, Sato S, Echigo T, et al. Up regulated expression of fractalkine/CX3CL1 and CX3CR1 in patients with systemic sclerosis. *Ann Rheum Dis*. 2005;64:21–28.
47. Chousterman BG, Boissonnas A, Poupel L, et al. Ly6Chigh monocytes protect against kidney damage during sepsis via a CX3CR1-dependent adhesion mechanism. *J Am Soc Nephrol*. 2016;27:792–803.
48. Dorfman MD, Krull JE, Douglass JD, et al. Sex differences in microglial CX3CR1 signalling determine obesity susceptibility in mice. *Nat Commun*. 2017;8:14556.
49. Cederblad L, Rosengren B, Ryberg E, et al. AZD8797 is an allosteric non-competitive modulator of the human CX3CR1 receptor. *Biochem J*. 2016;473:641–649.
50. Gonzalez-Mateo GT, Aroeira LS, Lopez-Cabrera M, et al. Pharmacological modulation of peritoneal injury induced by dialysis fluids: is it an option? *Nephrol Dial Transplant*. 2012;27:478–481.